

Using Mosaics in the Keck/3DFM Time Lapse Software

Rafal Norton
Keck Software Developer
rnorton@ece.neu.edu

Optical Science Laboratory
Center for Subsurface Sensing and Imaging Systems (CenSSIS)
Northeastern University

Date Created: January 17, 2008

Overview of the Mosaic Capabilities

For the purposes of this document, a mosaic is essentially a sequence of X,Y stage coordinates that have been either specified by the user or computed by the software. The 3DFM/Keck microscope is currently equipped with a Prior Pro-Scan II motorized translation stage which is what performs the actual mosaics.

The mosaic features are available for all of the current time lapse modes. The mosaic configuration window is invoked by clicking on the “Mosaic” menu in the “Time Lapse Series” application and selecting “Mosaic Configuration”.

During the selected time lapse, the stage visits each of these positions in the order that they appear in the “Positions” list (from top to bottom). At each stage position, the appropriate imaging operations for the selected time lapse are performed and after all the positions in the list have been visited, this constitutes one “round”.

- When using mosaics with any of the “slice” time lapse modes, the “Number of images” parameter is the number of mosaics to be taken, so instead of a single image, a group of images representing a mosaic is taken.
 - *For example, if 10 images are entered in the “Number of images” entry box, this will be 10 mosaic rounds. The time lapse will visit and image each position in the list (from top to bottom), wait the specified amount of time, then will visit and image the positions again from the top of the list down. This will repeat until 10 “rounds” have been completed.*
- When using mosaics with any of the “z-stack” time lapse modes, the “Number of steps” which is normally the number of images to take for each z-stack, becomes the number of mosaics to be taken for each z-stack.

The software currently provides two mosaic types, manual and pattern mosaics

- A manual mosaic is really a collection of arbitrary user-defined positions that will be visited and imaged in the order in which they appear in the list. These are useful when one is only interested in imaging discreet, arbitrary regions of a sample.

Refer to the section, “Creating a Manual Mosaic” for instructions.

- A pattern mosaic will image a contiguous region of a sample, in either a raster or a snake pattern.

Refer to the section, “Creating a Pattern Mosaic” for instructions.

Overview of the Mosaic Controls

- Select “Enable Mosaic” to begin using the controls of the “Mosaic Configuration” window. The current X,Y stage coordinates will be inserted into the “Positions” list.

The Positions Control

- This is the window’s primary control. It contains the list of stage positions that will be visited (and imaged) during the selected time lapse, and a set of buttons for working with them. Stage positions are always visited starting from the top of the list and going to the bottom; the “numbering” of the positions is irrelevant (i.e. if “P5” is higher in the list than “P0”, “P5” will be visited first, not “P0”).

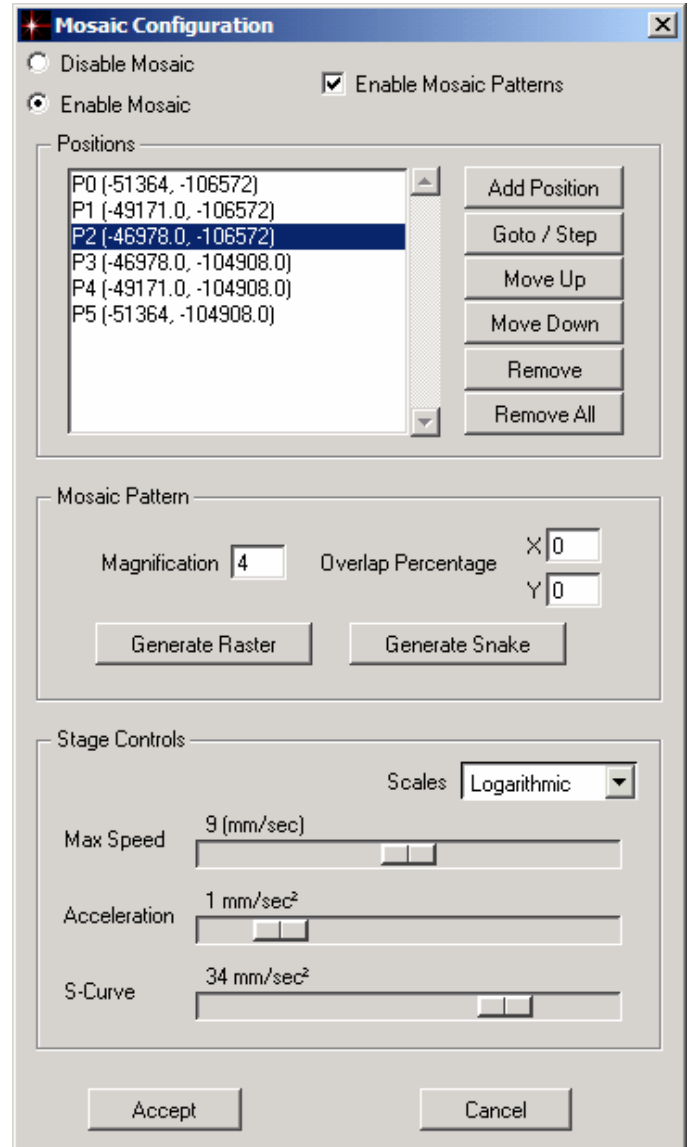
- **Add Position** adds the current X,Y stage coordinates to the end of the list.

For convenience, this action can also be invoked by pressing the ENTER/RETURN key.

- **Goto / Step** moves the stage to the currently highlighted position in the positions list, and then highlights the next position in the list. After the stage starts moving, this button changes to **Stop** to allow the user to stop the stage movement.

For convenience, this action can also be invoked by pressing the SPACE key. Pressing SPACE starts the movement; pressing it again while the stage is moving stops the stage.

- **Move Up** and **Move Down** move the selected position up or down in the list. Allows one to re-arrange the order of stage positions.
- **Remove** deletes the selected position from the list. This can also be invoked by pressing the DELETE key.
- **Remove All** deletes all positions from the list.



- Additionally, the Positions Control allows the stage to be moved using the dedicated arrow keys on the keyboard (i.e. not the ones on the numerical keypad). The stage's speed is controlled via the "Max Speed" slider in the "Stage Controls" section.
 - *To use the arrow keys to move the stage, keyboard and mouse focus must be set to the "Positions" control. This simply means either clicking on a position in the list, or just clicking anywhere in the positions list if there are no positions.*

The Mosaic Pattern Control

- This control is used to generate the raster or snake mosaic patterns. Refer to the section, "Creating a Pattern Mosaic" for details.

The Stage Controls

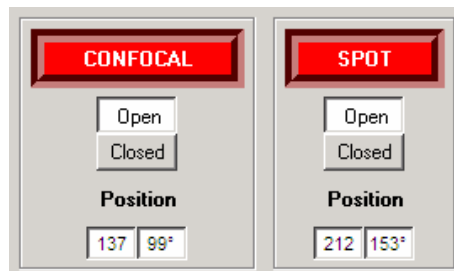
- "Max Speed" is the speed in millimeters per second the stage will move at. This affects the stage's speed when moving using the keyboard arrow keys and during the time lapse.
- The "Acceleration" parameter controls how quickly the stage accelerates (in millimeters per second squared) to and from its target speed ("Max Speed"). When the stage is instructed to move to an XY position, it will accelerate at the specified rate until it reaches the target speed. It will then decelerate from the target speed when it is halfway to its destination coordinates.
 - *To disable acceleration, set the "Acceleration" slider to 100. When acceleration is disabled, the stage will immediately start moving at its target speed ("Max Speed").*
 - *When moving the stage using the arrow keys, only the "Max Speed" parameter is in effect since it is not known at what stage coordinates movement will stop.*
- The "S-Curve" parameter provides fine tuning of the stage acceleration. It controls the rate of change (in millimeters per second squared) from the time the stage is stationary to the time the stage reaches the acceleration rate specified by the "Acceleration" slider.
- ❖ **Important:** Use these parameters carefully and reasonably especially when imaging aqueous samples. Sudden stops and motions can cause the sample (or parts of it) to move or "drift" thus degrading the quality and accuracy of the mosaic. Using acceleration and a low target speed can minimize the drifting of aqueous material. The actual stage motions can always be observed by having a real-time display of your sample, and stepping through each individual position. If the stage motions are too great, one can make adjustments and test them before starting the actual time lapse.
- ❖ When viewing a live display at higher magnifications, the minimum stage speed of 1 mm/sec may still create too large a movement. In that case, use the joystick located to the right of the Keck microscope to move the stage instead of the keyboard arrow keys.



Preparing to View Your Sample in Real-Time

- Make sure the appropriate shutters are open; otherwise, you will not see your sample. Start the “Shutter Control 1.1” program from its shortcut on the Keck desktop.

- *For one-photon, two-photon, or reflectance imaging, open the “Confocal” shutter by clicking on its “Open” button as shown to the right ...*
- *For brightfield, DIC, fluorescence, or epi-fluorescence imaging, open the “Spot” shutter by clicking on its “Open” button as shown to the right ...*



- You will also need to move a mirror into or out of the optical path depending on what kind of imaging you will be doing. You will see a rack with two mirrors directly below the microscope objectives. Start the “Zaber Positioner” program from its shortcut on the Keck desktop.
 - *For one-photon or reflectance imaging, click on the “Hot Mirror” button. You will hear the mirror rack moving. Wait for it to move into place. For two-photon imaging, click on the “Cold Mirror” and wait for it to move into place.*
 - *For brightfield, DIC, fluorescence, or epi-fluorescence, no mirror must be in the optical path. Click on the “Open” button and wait for the rack to move into place.*
- Start the appropriate live viewing application. You will need to see a live, real-time display of your sample in order to work with it.
 - *If you are acquiring brightfield, DIC, fluorescence, or epi-fluorescence images, start the “Spot Advanced” program from its shortcut on the Keck desktop. Then press the “Live” button on the program’s toolbar on the right side of the program window.*
 - *If you are acquiring confocal images (i.e. single-photon, two-photon, reflectance), start the “Confocal Subsystem” program from its shortcut on the Keck desktop. Then press “Grab”, and the live display of your sample will start momentarily.*
- After you have established a real-time display of your sample using either the “Spot Advanced” or the “Confocal Subsystem” programs, click back to the “Mosaic Configuration” window, and move it such that both it and the live image display are visible. You will also need to move the “Time Lapse Series” window so that it doesn’t obstruct your view of the real-time display. You may now begin working with your sample. Use the arrow keys on the keyboard to move the XY stage. You will see movement in the image display as you’re moving the stage. You may also use the joystick located to the right side of the microscope to move the XY stage.

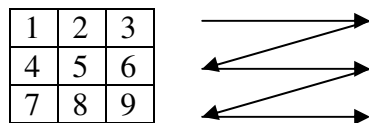
Creating a Manual Mosaic

- Click “Enable Mosaic” if you haven’t already done so.
- Prepare a real-time display of your sample as outlined in the section, “*Preparing to View Your Sample in Real-Time*”.
- Leave the “Enable Mosaic Patterns” box unchecked.
- Now use the arrow keys and/or joystick to move the stage. Find your objects of interest using the live image display, then click the “Add Position” button or press ENTER to add its stage coordinates to the list.
- Remember to test your mosaic setup before starting the time lapse. Use the “Goto / Step” button to step through your positions to ensure the stage moves using reasonable motions for what you will be imaging.
- During the time lapse, the images are NOT assembled or “stitched” together since this mode of operation is not created to image contiguous regions of a sample.

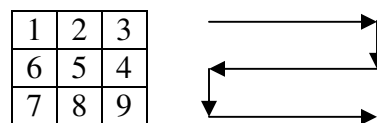
Creating a Pattern Mosaic

- Click “Enable Mosaic” if you haven’t already done so.
- Check the “Enable Mosaic Patterns” box. This will enable the “Mosaic Pattern” control.
- Prepare a real-time display of your sample as outlined in the section, “*Preparing to View Your Sample in Real-Time*”.
- With pattern mosaics, the user does not specify the actual stage positions; they are computed by the software. In order to compute a snake or raster pattern however, you must provide two stage positions; the upper left and the lower right boundaries of the region of interest. Move the stage using the keyboard arrow keys and/or the joystick and add those positions to the list.
- Enter the magnification you are using, and press “Generate Raster” or “Generate Snake” depending on which pattern of movement you prefer.
 - *It is generally recommended to use the “Snake” pattern especially when imaging aqueous samples. The snake pattern uses smaller movements which reduce the “drifting” of aqueous/liquid material while the mosaic is being performed.*

The stage motions of the snake and raster patterns are illustrated below using a 3x3 mosaic.



Raster Pattern Motion



Snake Pattern Motion

- *You may optionally specify overlap percentages in the X and/or Y directions. Overlap controls how much of an image appears in the next one in the mosaic sequence and is used to compensate for movements of the sample that may have occurred while the mosaic was being performed.*
- The software will now generate the stage positions that will image the region of interest using the selected pattern and will place them in the “Positions” list.
- ❖ **Important:** Do not edit the generated position list in any way! Doing so will cause incorrect assembling of the images in the mosaic sequence. Do not delete, add any new positions or rearrange the order of the generated list. To obtain a new list of positions using different parameters, and/or a different region of interest, you must first delete the existing ones (via “Remove All”), then repeat all the steps in this section.

- Remember to test your mosaic setup before starting the time lapse. Use the “Goto / Step” button to step through your positions to ensure the stage moves using reasonable motions for what you will be imaging.
- Each mosaic will be “assembled” and placed into the folder named “stitched” located in the same folder as your image files.
- It is highly recommended to also perform a “test run” first before starting the time lapse.
 - *For “slice” time lapse operations, enter 1 for the “Number of images”, and 0 for the “wait time” parameters.*
 - *For “z-stack” time lapse operations specify 1 for the “Number of z-stacks”, 1 for the “Number of steps”, 1 for the “Step size”, and 0 for the “wait time” parameters.*
 - *Start the time lapse. After it has finished, examine the assembled mosaic image located in the “stitched” folder to be sure the result is acceptable before starting the time lapse.*